# The Role of Molecular Diagnostics in the Management of Patients with Gliomas

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Abstract: The revised World Health Organization (WHO) classification of tumors of the central nervous system of 2016 combines biology-driven molecular marker diagnostics with classical histological cancer diagnosis. Reclassification of gliomas by molecular similarity beyond histological boundaries improves outcome prediction and will increasingly guide treatment decisions. This change in paradigms implies more personalized and eventually more efficient therapeutic approaches, but the era of molecular targeted therapies for gliomas is yet at its onset. Promising results of molecularly targeted therapies in genetically less complex gliomas with circumscribed growth such as subependymal giant cell astrocytoma or pilocytic astrocytoma support further development of molecularly targeted therapies. In diffuse gliomas, several molecular markers that predict benefit from alkylating agent chemotherapy have been identified in recent years. For example, co-deletion of chromosome arms 1p and 19q predicts benefit from polychemotherapy with procarbazine, CCNU (lomustine), and vincristine (PCV) in patients with anaplastic oligodendroglioma, and the presence of 1p/19q co-deletion was integrated as a defining feature of oligodendroglial tumors in the revised WHO classification. However, the tremendous increase in knowledge of molecular drivers of diffuse gliomas on genomic, epigenetic, and gene expression levels has not yet translated into effective molecular targeted therapies. Multiple reasons account for the failure of early clinical trials of molecularly targeted therapies in diffuse gliomas, including the lack of molecular entry controls as well as pharmacokinetic and pharmacodynamics issues, but the key challenge of specifically targeting the molecular backbone of diffuse gliomas is probably extensive clonal heterogeneity. A more profound understanding of clonal selection, alternative activation of oncogenic signaling pathways, and genomic instability is warranted to identify effective combination treatments and ultimately improve survival.

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**The Role of Molecular Diagnostics in the Management of Patients with Gliomas**

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Illustrating material:

Table 1 – Gliomas in the 2016 WHO classification of primary tumors of the central nervous system

Figure 1 – Overview of molecular subtypes of diffuse gliomas

## Opinion statement

The revised World Health Organization (WHO) classification of tumors of the central nervous system of 2016 combines biology driven molecular marker diagnostics with classical histological cancer diagnosis. Reclassification of gliomas by molecular similarity beyond histological boundaries improves outcome prediction and will increasingly guide treatment decisions. This change in paradigms implies more personalized and eventually more efficient therapeutic approaches, but the era of molecular targeted therapies for gliomas is yet at its onset. Promising results of molecularly targeted therapies in genetically less complex gliomas with circumscribed growth such as subependymal giant cell astrocytoma or pilocytic astrocytoma support further development of molecularly targeted therapies. In diffuse gliomas, several molecular markers that predict benefit from to alkylating agent chemotherapy have been identified in recent years. For example, co-deletion of chromosome arms 1p and 19q predicts benefit from to polychemotherapy with procarbazin, CCNU (lomustine) and vincristine (PCV) in patients with anaplastic oligodendroglioma, and the presence of 1p/19q co-deletion was integrated as a defining feature of oligodendroglial tumors in the revised WHO classification. However, the tremendous increase in knowledge of molecular drivers of diffuse gliomas on genomic, epigenetic and gene expression levels has not yet translated into effective molecular targeted therapies. Multiple reasons account for the failure of early clinical trials of molecularly targeted therapies in diffuse gliomas, including the lack of molecular entry controls as well as pharmacokinetic and pharmacodynamics issues, but the key challenge of specifically targeting the molecular backbone of diffuse gliomas is probably extensive clonal heterogeneity. A more profound understanding of clonal selection, alternative activation of oncogenic signaling pathways and genomic instability is warranted to identify effective combination treatments and ultimately improve survival.

## Introduction

Gliomas are a histologically and molecularly heterogenous group of tumors that are thought to derive from neuroglial progenitor cells and that account for approximately 27% of all primary brain tumors and for 80% of malignant primary brain tumors [1]. Histologically, the WHO classification of tumors of the central nervous system distinguishes astrocytomas, oligodendrogliomas and ependymomas, and assigns WHO grades I-IV with respect to the grade of malignancy [2, 3]. By convention, WHO grade I and II gliomas were referred to as low-grade and grades III and IV as high-grade gliomas. Low-grade gliomas comprised all gliomas with circumscribed growth, and these are rare and molecularly less complex than diffuse gliomas. The vast majority of gliomas are, however, characterized by diffuse growth and heterogenous molecular patterns, and tremendous progress of molecular profiling array technologies has yielded genomic, epigenetic and gene expression landscapes that have refined the understanding and classification of biologically distinct glioma subtypes beyond histology [4-6] (Figure 1). These advances were acknowledged with the 2016 revision of the WHO classification of tumors of the central nervous system, which includes novel classes of diffuse gliomas based on genomic features (Table 1). Molecular diagnostics trump discordant histological results and thereby increase diagnostic accuracy and prognostic yield compared to previous histology based classifications [3]. Accordingly, the division into low grade and high grade gliomas is outdated and should be abandoned.

In parallel to these advances in classifying gliomas, the identification of key drivers of malignancy led to novel therapeutic approaches to target the molecular machinery of gliomas. Success of such targeted therapies in the molecularly less complex gliomas with circumscribed growth support this principle, but the extensive intratumoral heterogeneity of diffuse gliomas poses major challenges: First, large numbers of

molecular alterations in each tumor complicate the differentiation between passenger and driver events. Second, targeting a single driver alteration may be ineffective due to compensating pathways that are active in parallel or can be readily activated. Third, successful targeting of a tumor cell population may be compensated by clonal selection of another population that does not bear the targeted alteration. Therefore, a profound understanding of the molecular events that initiate and drive the progression of gliomas is crucial for the development of molecularly targeted therapies, because ultimately only targeting of key disease drivers that are shared by all cells within the tumor, or combination therapies can overcome treatment resistance.

Innovative trial designs have been suggested, such as umbrella trials that incorporate molecular profiling at study entry and the subsequent choice of an individually tailored therapy that matches the molecular profile of the tumor [7]. The scope of such trial designs is exploratory, thus underscoring the necessity to include repeat biopsies to enable the assessment of mechanisms of escape or resistance to molecular targeted therapies. However, the choice of tested molecular alterations in such trial designs is subject to expert opinions and availability of valid inhibitors that, amongst others, match the pharmacokinetic issues posed by the blood brain barrier. Technological advances such as deep sequencing and single-cell array approaches in tissues derived from sequential biopsies or surgeries will shed further light into the mechanisms underlying therapy resistance and malignant progression, albeit the prospective applicability of such large-scale arrays for clinical decision making or as molecular entry controls for clinical trials is still limited by logistic issues such as the requirement of extensive financial, human and technological resources, as well as a lack of consensus on data interpretation.

This review will summarize the current knowledge of the molecular background of gliomagenesis, the applicability of molecular marker diagnostics derived from this knowledge as well as current and future implications for precision medicine in gliomas.

**Gliomas with circumscribed growth**

Improved understanding of the molecular basis of gliomas with circumscribed growth yielded promising results of molecularly targeted therapies. For example, subependymal giant cell astrocytomas (SEGA) are driven by mutations in either of two tumor suppressor genes, tuberous sclerosis complex (*TSC*)*-1* and *-2* that encode hamartin and tuberin, respectively. Loss of either protein yields disinhibition of cell growth signaling through mammalian target of rapamycin (mTOR) [8], and the mTOR inhibitor everolimus is effective against SEGA [9, 10].

Pilocytic astrocytoma is likewise considered a single pathway disease that is driven by the mitogen-activated protein kinase (MAPK) pathway due to mutations in the fibroblast growth factor receptor 1 (*FGFR1*) or neurotrophic receptor tyrosine kinase 2 (*NTRK2*) genes, or due to tandem duplications in a v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) fusion gene [11, 12] and an early phase study of the MAPK inhibitor selumetinib in pilocytic astrocytoma has recently completed accrual (NCT01089101).

Pleomorphic xanthoastrocytomas are rare and less well characterized compared to other glioma entities. Particularly the mechanisms underlying the rare instances of malignant progression remain unclear. However, activating $BRAF^{V600E}$ mutations are present in more than half of pleomorphic xanthoastrocytomas [13] and recent reports of response to vemurafenib in heavily pretreated patients with recurrent pleomorphic xanthoastrocytomas suggest that the presence of the $BRAF^{V600E}$ mutation lends a rationale for salvage treatment with drugs like vemurafenib [14, 15], but no prospective trials are ongoing to further explore the role of $BRAF^{V600E}$ as a putative molecular target in pleomorphic xanthoastrocytomas.

**Ependymomas**

Low-grade ependymomas are characterized by circumscribed growth patterns, but diffuse growth and multifocal progression occur frequently in the course of disease. Assessment of the v-rel avian reticuloendotheliosis viral oncogene homolog A *(RELA)–C11orf95* fusion gene has recently entered clinical routine, because *RELA* fusion-positive ependymomas have been included in the revised WHO classification as an entity with high risk for rapid and diffuse growth at diagnosis or during the disease course [3, 16]. A total of 9 molecular ependymoma subtypes have been defined, including a second high-risk subtype designated posterior fossa A (PF-A). PF-A ependymomas comprise the vast majority of posterior fossa ependymomas, but a driver alteration of this probably epigenetically driven disease has not yet been identified [17]. The standard of care in ependymomas comprises maximum safe resection and post-operative radiotherapy [18, 19]. In consideration that approximately half of all patients with ependymomas are children and that maximum resection may indeed be curative in a substantial fraction of patients, the timing of radiotherapy is a key aspect of the mangement of ependymomas, but probably radiotherapy should not be delayed in PF-A and *RELA* fusion-positive high-risk patients. The role of chemotherapy for the treatment of ependymomas has remained elusive and mostly confined to the recurrent setting [19]. Ongoing trials assess carboplatin-based regimens with and without bevacizumab (NCT01295944, NCT01088035), everolimus (NCT02155920) as well as a combination of temozolomide and the dual epithelial growth factor receptor (EGFR) inhibitor lapatinib (NCT00826241), which is directed against the EGFR isotypes Erb1 and Erb2 (HER2/neu), but molecularly targeted therapies taking the recent definitions of ependymoma subtypes into account have not yet been launched. Molecularly guided trials are complicated by the overall low frequency of ependymomas and further restrictions implicated by the apparent molecular diversity.

## Diffuse oligodendroglial and astrocytic gliomas

Joint efforts of the scientific community in characterizing the complex biology of diffuse gliomas culminated in The Cancer Genome Atlas Research Network (TCGA) and recent integration of information on exome sequences, DNA copy numbers, epigenetics, as well as messenger RNA, microRNA and protein expression identified prognostic subgroups of diffuse gliomas that were defined more accurately than by histology [6, 20, 21] (Figure 1). Molecular markers that represent a surrogate excerpt of these complex analyses are warranted to enable the translation of knowledge derived from these complex large-scale analyses into clinical practice (Table 1). Most current studies come up with a simplified approach of characterizing three major classes of gliomas, defined by isocitrate dehydrogenase (IDH) mutation plus 1p/19q codeletion, IDH mutation without 1p/19q codeletion, or IDH wildtype status [20, 22-25]. A classification of 5 prognostic adult glioma subgroups has been proposed based on the presence of *IDH* mutations, 1p/19q co-deletions and telomerase reverse transcriptase (*TERT*) promoter mutations [26], and independently of this classification, *MGMT* promoter methylation has emerged as a predictive marker for benefit from temozolomide chemotherapy in elderly patients with glioblastoma [27, 28].

***IDH* mutations.** The integration of *IDH* mutations into the revised WHO classification as a primary categorizing molecular alteration of diffuse gliomas has underpinned a change in paradigms from a histology-based towards a biology-based categorization of gliomas [3]. The conceptual novelty of the 2016 WHO classification is exemplified by the close and causal association of mutant IDH with a distinct epigenetic pattern designated glioma-CpG island methylator phenotype (G-CIMP) [29, 30]. Oncogenesis studies identified *IDH* mutations as early and possibly initiating events during

gliomagenesis and demonstrated that the G-CIMP pattern is preserved throughout malignant progression of *IDH* mutated gliomas from WHO grades II through IV [5, 31]. The vast majority of histological WHO grade II/III gliomas harbor *IDH* mutations, but only about 10% of glioblastomas [32, 33]. The histological appearance of *IDH* mutated versus wild-type glioblastomas is largely identical, but *IDH* mutated glioblastomas are associated with younger age at diagnosis and a better prognosis. An antibody to specifically detect the most common *IDH* mutation in gliomas *IDH1*$^{R132H}$ by immunohistochemistry is commercially available [34] and additional sequencing to detect other *IDH1/2* mutations is recommended in patients with WHO grade II or III gliomas or young adults with glioblastoma whose tumors show negative immunohistochemistry [35]. To date, the detection of *IDH* mutations has no direct therapeutic implications, but refines the prognostic accuracy of the WHO classification.

**Co-deletion of chromosome arms 1p and 19q.** According to the 2016 WHO classification, co-deletion of chromosome arms 1p and 19q is a defining feature of oligodendroglial tumors, whereas 1p/19q non-co-deleted tumors are classified as astrocytomas [3]. Notably, this molecular definition of neuroglial lineage association has outdated the diagnosis of a mixed oligoastrocytic entity, the histological diagnosis of which has been debated because of inter-observer variations and which, according to the revised WHO classification, may now only be diagnosed in patients that lack assessment of 1p/19q status [3, 36]. 1p/19q co-deleted oligodendrogliomas constitute a sub-group of *IDH* mutated gliomas that commonly harbor additional activating mutations in the promoter region of *TERT*. In contrast, oncogenic mutations in the tumor suppressor gene *TP53* and in the methylome organizer alpha thalassemia/mental retardation syndrome X-linked (*ATRX*) are primarily found in *IDH* mutated astrocytomas and associated with less favorable prognosis [26, 36, 37]. Fluorescent *in situ* hybridization (FISH) and microsatellite analysis for loss of heterozygosity are commonly applied to assess 1p/19q status in clinical routine. The

prognosis of 1p/19q co-deleted gliomas is favorable and malignant progression to glioblastoma is rare. Besides diagnostic and prognostic implications, a predictive role of 1p/19q co-deletion for benefit from the addition of polychemotherapy with procarbazine, CCNU/lomustine and vincristine (PCV) to radiotherapy was identified in long-term analyses of two phase III trials in patients with anaplastic oligodendroglial tumors at a median follow-up time of more than ten years [38, 39]. Less toxic regimens were commonly utilized in clinical practice by the time these results have re-defined PCV as the standard of care for 1p/19q co-deleted tumors and the phase III CODEL trial is currently exploring whether PCV may be substituted by temozolomide to reduce toxicity, without compromising survival (NCT00887146).

**Mutations of the telomerase reverse transcriptase (*TERT*) promoter.** *TERT* promoter mutations are present in most diffuse gliomas and confer poor prognosis in patients with *IDH* wild-type, WHO grade II/III diffuse gliomas [26]. This is of particular relevance for the management of patients with WHO grade II diffuse gliomas, because watchful waiting strategies are considered feasible in patients with favorable prognostic features, including age < 40, tumor diameter < 6 cm, lack of midline crossing and lack of neurological deficit other than an brain tumor-associated epilepsy [40-42], but such strategies are probably not adequate in the poor prognosis subgroup of TERT mutated, IDH wild type diffuse gliomas.

**Hypermethylation of the O6-methylguanyl DNA methyltransferase (*MGMT*) promoter.** The DNA repair protein MGMT counteracts DNA damage conferred by alkylating agent chemotherapy. Transcriptional silencing of the *MGMT* gene due to promoter hypermethylation occurs almost invariably in G-CIMP positive, *IDH* mutated diffuse gliomas, as opposed to approximately 50% of G-CIMP negative, *IDH* wild-type diffuse gliomas [26, 43]. *MGMT* methylation status has a pivotal role for clinical decision-making, e.g., in elderly glioblastoma patients, but *MGMT* does not segregate

glioma subtypes with distinct gene ontologies. Consequently, *MGMT* methylation status was not included as a segregating feature in the revised WHO classification. Of note, different technical approaches and a lack of consensus on data interpretation render *MGMT* status assessments prone to variability [44]. *MGMT* promoter methylation was strongly associated with benefit from chemotherapy with temozolomide in the trial carried out by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) that defined adjuvant temozolomide as the standard of care for newly diagnosed glioblastoma [45, 46]. In the elderly setting, the superiority of the combination of temozolomide and radiotherapy over radiotherapy alone has been confirmed [47]. Elderly patients with diffuse gliomas not considered candidates for combined modality treatment are commonly treated with monotherapy regimens [48]. Two phase III trials in elderly patients with mostly glioblastomas and a smaller fraction of anaplastic astrocytomas defined a predictive role of *MGMT* methylation for response to temozolomide versus radiotherapy to guide decision making at primary diagnosis [27, 28]. The strong association of *MGMT* methylation with benefit from temozolomide in glioblastoma is retained at recurrence, thus making rechallenge with temozolomide a viable option for patients with *MGMT* methylated glioblastoma and prolonged progression-free survival with first-line radiochemotherapy [49]. Changes of *MGMT* promoter methylation status at recurrence have not been observed in studies comparing matched tissue samples [50]. Interestingly, the prognostic value of *MGMT* methylation was similar among patients receiving radiotherapy versus chemotherapy in subgroup analyses of two phase III trials in patients with anaplastic gliomas [51, 52], albeit more global DNA methylation analyses suggest a predictive role of methylation of two specific CpG sites in the *MGMT* promoter for benefit from PCV [53]. However, the number of samples in this cohort was small and warrants confirmatory analyses. Overall, the role of *MGMT* promoter methylation status in WHO grade II and grade III gliomas remains a matter of controversy.

**Histone H3.3 (*H3F3A*) K27 and G34 mutations.** Point mutations in the 2 critical aminoacids K27 and G34 of the gene encoding histone H3.3 define *IDH* wild-type glioma subtypes in children and adolescents with G-CIMP negative gene methylation patterns that arise from distinct anatomical compartments [21, 54]. K27 and G34 mutations are mutually exclusive and strongly associated with mutations of the tumor suppressor gene *TP53* [21]. The strong anatomical and molecular association prompted the inclusion of *H3F3A*$^{K27M}$ mutant diffuse midline glioma as a novel entity to the 2016 WHO classification [3]. K27 mutations confer a poor prognosis, which may in part be attributed to the poorer surgical accessibility of midline tumors, but to date, no therapeutic implications derive from the presence of *H3F3A* mutations.

**Molecular subtypes derived from integrated large-scale analyses.** The complexity of diffuse gliomas is not fully captured by marker-based sub-classification. Integrated gene expression and genomic analyses defined 4 glioblastoma subtypes that were associated with distinct genomic alterations and that were termed neural, proneural, classical and mesenchymal based on similarities to previously defined developmental gene expression signatures [55]. Unsupervised cluster analyses of subsequent independent datasets did not reproduce the segregation of the neural subtype signature as a distinct entity [21, 56], but further integration of methylation data defined a total of 6 subtypes, including 4 that clustered with proneural gene expression and are more common in children or young adults [21]. *IDH* mutated glioblastomas were the only proneural glioblastomas to confer a favorable prognosis, whereas the prognostic less favorable subtypes with proneural gene expression were characterized by *H3F3A* mutations in the codons of the critical amino acids K27 or G34, or by amplification of the gene encoding platelet derived growth factor receptor A (*PDGFRA*). *PDGFRA* amplified glioblastomas were designated receptor tyrosine kinase (RTK) I, as opposed to the non-proneural RTK II subtype that is characterized

by a classical gene expression pattern and amplification of the receptor tyrosine kinase *EGFR*, and both RTK I and RTK II subtypes are furthermore associated with deletions of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) tumor suppressor gene and poor prognosis. The mesenchymal subtype shows a lower rate of copy-number alterations and no characteristic mutations, but likewise confers poor prognosis [21]. More recently, the applicability of these glioblastoma-based classifications to the entire histological spectrum of diffuse oligodendroglial and astrocytic diffuse gliomas was confirmed and two further prognostic subgroups were identified: (i) A small subgroup of *IDH* mutated astrocytic gliomas with poor prognosis that do not exhibit the characteristic G-CIMP gene methylation pattern, but are instead characterized by cell cycle activation due to *CDKN2A* deletions and cyclin dependent kinase 4 (*CDK4*) amplifications, and (ii) a small subgroup *IDH* wild-type WHO grade II/III astrocytic and oligodendroglial tumors in mostly young adults with favorable prognosis that share epigenetic and genomic features of pilocytic astrocytomas [6]. Such large-scale analyses rely on data derived from entire tissue samples, but another level of complexity is added by the extensive spatial and temporal molecular heterogeneity of gliomas [4, 5, 31].

**Avenues and challenges of molecular targeted therapies in diffuse gliomas**

The perspective of specifically targeting disease drivers in diffuse gliomas evoked high hopes for future effective treatments, but to date such attempts have failed beyond early phase studies. The paramount reason for this failure is the molecular complexity and heterogeneity of diffuse gliomas, but a broad range of factors that can be overcome by careful planning of clinical trials have had their share, too. For example, pharmacokinetic and pharmacodynamics issues have complicated early trials of EGFR inhibition in glioblastoma: The glioma-specific *EGFRvIII* deletion

mutation that is present in half of all *EGFR* amplified glioblastomas yields constitutive, ligand-independent receptor activity that is not targeted by the EGFR inhibitors erlotinib or gefitinib, whereas brain penetration of the EGFRvIII inhibitor lapatinib is limited [57]. Another challenge in establishing molecular targeted therapies is posed by the choice of hypothesis-based or empirical molecular entry controls to chose patients that will potentially benefit from a trial drug. For example, the mammalian target of rapamycin (mTOR) inhibitor temsirolimus (CCI-779) failed to improve outcome in two separate phase II trial cohorts, whereas response was associated with phosphorylation of mTORSer2448 in newly diagnosed *MGMT* unmethylated glioblastoma [58] and with phosphorylation of the mTOR target S6-kinase in recurrent glioblastoma [59], and efficacy studies further exploring mTOR inhibition in glioblastoma patients pre-selected for these molecular markers are currently probably not underway.

Finally, chosing the right target poses the most difficult challenge to clinical trials that aim at specifically targeting the molecular machinery of cancers. In consideration of the extensive vascularity of glioblastomas, targeting signaling through the key molecular signaling molecule vascular endothelial growth factor (VEGF) was explored extensively, but a total of six phase III trials in newly diagnosed and recurrent glioblastoma failed to demonstrate an overall survival benefit from anti-angiogenic therapy targeting either the VEGF or other putative angiogenic pathways [60-65]. Molecular analyses have however identified the proneural gene expression signature as a potential molecular marker for the benefit of an adjunct of the anti-VEGF antibody bevacizumab to standard chemoradiotherapy in newly diagnosed glioblastoma [66], but a confirmatory trial that applies this signature prospectively is currently not planned and the opportunity to validate this finding in the RTOG 0825 trial is pending. Considering the prominent role of epigenetic mechanisms in the pathophysiology of diffuse gliomas, measures to induce epigenetic reshaping were successfully applied in preclinical studies, including a small molecule inhibitor of

mutant *IDH* [67] and an inhibitor of the K27 demethylase JMJD3, which yielded K27 hypermethylation and potent anti-tumor activity in a pre-clinical model of H3 K27M–mutant diffuse midline glioma [68], but clinical trials to test these inhibitors await to be completed.

Immunotherapy approaches have recently moved into the focus of novel developments in oncology: In patients with metastatic melanoma and non-small-cell lung cancer, monoclonal antibodies directed against the immunosuppressive T-cell receptors programmed death protein (PD)1 (nivolumab, pembrolizumab) or cytotoxic T-lymphocyte antigen (CTLA) 4 (ipilimumab) have proven a successful strategy to improve survival by promoting innate immune-mediated anti-tumor activity [69-73]. The efficacy of nivolumab is currently being assessed in newly diagnosed glioblastoma as a substitute for temozolomide in patients with an unmethylated *MGMT* promoter in the phase III trial CheckMate 498 (NCT02617589), and as an adjunct to temozolomide in patients with a methylated *MGMT* promoter in the phase II trial CheckMate 548 (NCT02667587). Furthermore, the phase III trial CheckMate 143 assessed the efficacy of nivolumab versus bevacizumab in patients with recurrent glioblastoma (NCT02017717).

Vaccination against tumor antigens is another immunotherapy approach that is being explored in glioblastoma. The double-blinded phase II ICT-107 trial explored the safety and efficacy of a vaccine consisting of autologous dendritic cells that were pulsed with six tumor-associated peptides to trigger an anti-tumor T-cell response in newly diagnosed glioblastoma. The adjunct of this vaccine to standard chemoradiotherapy prolonged progression-free survival by 2.4 months without relevant safety issues [74] and therefore led to a similar phase III trial (NCT02546102). The DCVax trial is a phase III trial that assesses the efficacy of vaccination with dendritic cells that were pulsed with patients' tumor lysates (NCT00045968). Using peptides for vaccination that occur specifically in tumor cells due to coding mutations potentially prevents cross reactivity of an elicited immune

response against normal host cells and renders immune tolerance less likely. However, due to the multi-branched ontology of diffuse gliomas, only few mutations that were acquired early during gliomagenesis are present in all clones [5], such as mutant IDH which could be targeted successfully in a pre-clinical glioma model [75]. In contrast, clonal selection is a likely mechanism of resistance to vaccines that aim to elicit immune responses against mutations acquired later during gliomagenesis, such as the *EGFRvIII* deletion. This limitation may underly the lack of efficacy of vaccination with the EGFRvIII-specific peptide rindopepimut as an adjunct to standard chemoradiotherapy in newly diagnosed EGFRvIII-positive glioblastoma (NCT01480479), albeit the same vaccine appeared to prolong survival as an adjunct to the anti-angiogenic and probably pro-immunogenic anti-VEGF antibody bevacizumab in the phase II ReACT trial in patients with recurrent glioblastoma [76]. The term adoptive T-cell transfer refers to an immunotherapy approach that exploits the *in vitro* expansion or genetic engineering of T-cells directed against tumor-associated or tumor-specific peptides, and chimeric antigen-receptor (CAR) T-cells are genetically engineered cells that furthermore circumvent the need for co-stimulatory molecules by linking antigen-binding domains directly to intracellular signaling domains that trigger T-cell activation [77]. A phase I trial in glioblastoma patients utilizing CAR T-cells directed against Her2 is ongoing (NCT01109095) and another trial utilizing CAR T-cells directed against EGFRvIII has suspended recruitment (NCT01454596).

In summary, the current quest for effective and feasible treatment modalities and targets is yet at the onset of the personalization of treatments in patients with diffuse gliomas. Probably only combination treatments guided by molecular fingerprints that predict or capture clonal heterogeneity will be able to overcome resistance, but a plethora of issues including logistic feasibility and toxicity will have to be resolved along this development.

**Figure 1. Overview of molecular subtypes of diffuse gliomas.** The layered classification included in the 2016 WHO classification is depicted in red. Diffuse gliomas are segregated by *IDH* mutation status. 1p/19q codeletion marks oligodendrogliomas and the *H3F3A*$^{K27}$ mutation marks diffuse midline gliomas. These molecular assessments trump histological tumor grades II-IV. Other less robust genetic and genomic (green), epigenetic (blue) and gene expression (yellow) patterns that have not been included in the WHO classification have been defined to further segregate diffuse gliomas based on molecular features. Relative prognosis and age associations are depicted at the bottom.

**Table 1. Gliomas in the 2016 WHO classification of primary tumors of the central nervous system [3].**

| Entity | Histological WHO grade | Median overall survival | First line therapy | Comments |
|---|---|---|---|---|
| | | | | |
| **Diffuse astrocytic and oligodendroglial tumours** | | | | |
| | | | | |
| Diffuse astrocytoma, IDH mutant | II | > 10 years [78] | Watchful waiting [40] or RT→PCV [79] or (TMZ)/RT→TMZ per extrapolation from WHO grade III gliomas [80] | The previous term fibrillary astrocytoma is discouraged, because fibrillary morphology is the classical appearance and not a variant. |
| Gemistocytic astrocytoma, IDH mutant | II | < 4 years [81] | RT→PCV [79] or (TMZ)/RT→TMZ per extrapolation from WHO grade III gliomas [80] | Variant of diffuse astrocytoma. Higher incidence of progression to IDH-mutant anaplastic astrocytoma and glioblastoma has been reported [81]. Survival times including IDH-mutation status have not been reported. Watchful waiting strategies may not be adequate, if less favorable prognosis is confirmed in IDH mutated gemistocytic astrocytoma. |
| Diffuse astrocytoma, IDH wild-type | II | > 5 years [79] | RT→PCV [79] or TMZ/RT→TMZ per extrapolation from IDH wild-type glioblastoma [46] | Rare, provisional entity comprising a variety of molecular subtypes mostly resembling IDH wild-type glioblastomas [20, 82]. |

| | | | | |
|---|---|---|---|---|
| | | | | Watchful waiting strategies are probably not adequate.<br>Survival times have not been reported. |
| Diffuse astrocytoma, NOS | II | 4-5 years [1] | RT→PCV [79] or (TMZ)/RT→TMZ per extrapolation from WHO grade III gliomas [80] | |
| | | | | |
| Anaplastic astrocytoma, IDH mutant | III | 5-10 years [33, 78] | (TMZ)/RT→TMZ [80] | No population-based survival data reported. |
| Anaplastic astrocytoma, IDH wild-type | III | 2-4 years [26, 33, 83] | TMZ/RT→TMZ per extrapolation from IDH wild-type glioblastoma [46], possibly RT alone in MGMT unmethylated patients [84] | Molecularly resembling IDH wild-type glioblastoma, *TERT* promoter mutations confer poor prognosis [20, 82].<br>No population-based survival data reported. |
| Anaplastic astrocytoma, NOS | III | 1-2 years [1] | (TMZ)/RT→TMZ [80] | |
| | | | | |
| Glioblastoma, IDH wild-type | IV | 11-15 months [33, 85, 86] | TMZ/RT→TMZ [46, 47], > 65-70 years RT (MGMT unmethylated), or TMZ (MGMT methylated), if combination therapy is not deemed feasible [27, 28] | Histological variants: Giant cell glioblastoma, gliosarcoma, epitheloid glioblastoma |
| Glioblastoma, IDH mutant | IV | 2-3 years [33, 85] | (TMZ)/RT→TMZ per extrapolation from IDH mutant anaplastic astrocytoma [80] | |
| Glioblastoma, NOS | IV | < 1 year [1, 86] | See above | |
| | | | | |
| Diffuse midline glioma, H3-K27M mutant | IV | < 1 year [21] | TMZ/RT→TMZ per extrapolation from IDH wild-type glioblastoma [46, 47] | No population-based survival data reported. |

| | | | | |
|---|---|---|---|---|
| Oligodendroglioma, IDH mutant and 1p/19q codeleted | II | > 10 years [79] | Watchful waiting [40] or RT→PCV [79] | No population-based survival data reported. |
| Oligodendroglioma, NOS | II | > 10 years [1, 87] | Watchful waiting [40] or RT→PCV [79] | |
| | | | | |
| Anaplastic oligodendroglioma, IDH mutant and 1p/19q codeleted | III | > 10 years [38, 39, 83] | RT→PCV [38, 39] | No population-based survival data reported. |
| Anaplastic oligodendroglioma, NOS | III | 5-7 years [1, 83] | RT→PCV [38, 39] | |
| | | | | |
| Oligoastrocytoma, NOS | II | < 7 years [81] | Watchful waiting [40] or RT→PCV [79] per extrapolation from 1p/19q codeleted oligodendroglioma | Large interobserver variability of histological diagnosis [36, 88]. |
| Anaplastic oligoastrocytoma, NOS | III | > 5 years [89] | RT→PCV [38, 39] per extrapolation from 1p/19q codeleted anaplastic oligodendroglioma | Large interobserver variability of histological diagnosis [36, 88]<br>No population-based survival data reported. |
| | | | | |

NOS, not otherwise specified (Histological diagnosis in cases where molecular data have not been fully assessed)

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